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Salt-induced hydrogen peroxide is involved in modulation of antioxidant enzymes in cotton

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ABSTRACT

Salt severely restricts cotton (*Gossypium hirsutum*) growth and production. The present study was undertaken to study the effect of salt-induced hydrogen peroxide (H_2O_2) on antioxidant enzymes in cotton. NaCl treatment or exogenous H_2O_2 was used to investigate the relationship between H_2O_2 content and levels of antioxidant enzymes including superoxide dismutase (SOD), ascorbate peroxidase (APX), peroxidase (POD), and catalase (CAT), as well as the transcriptional levels of corresponding genes. H_2O_2 content increased within 24 h following 200 mmol L^{-1} NaCl treatment. Both NaCl-induced and exogenous H_2O_2 increased the activity of antioxidant enzymes including APX and SOD and upregulated the transcriptional levels of *GhcAPX1*, *GhFeSOD*, and *GhchlCSD*. These increased activities and upregulated transcriptional levels were inhibited when the salt-induced H_2O_2 was scavenged by NAC. These results indicate that salt-induced H_2O_2 as a second signaling messenger modulates APX and SOD activities by regulating the transcription levels of corresponding genes, alleviating oxidative stress, and increasing salt tolerance in cotton.

1. Introduction

In arid and semiarid regions of the world, increased soil salinity impairs plant productivity in many different ways [1]. One of the damages induced by salt is the increase in reactive oxygen species (ROS) such as superoxide (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical ($^{\cdot}OH$) [2]. Higher plants produce ROS, which can be neutralized by intracellular antioxidants under normal conditions, but excessive accumulation during stress conditions can cause oxidative stress [2,3] and severely disrupt normal metabolism by peroxidation of membrane lipids [4,5], protein destruction [6], and nucleic acid mutation [4,7]. Such oxidative damages may result in diseases and degenerative processes. To maintain growth and

Abbreviations: SOD, superoxide dismutase; CAT, catalase; POD, peroxidase; APX, ascorbate peroxidase; ROS, reactive oxygen species; H_2O_2, hydrogen peroxide; O_2^-, superoxide anion; OH, hydroxyl radical; *GhFeSOD*, *G. hirsutum* FeSOD gene; *GhchlCSD*, *G. hirsutum* chloroplast Cu/ZnSOD gene; *GhcAPX1*, *G. hirsutum* cytosolic APX1 gene; *GhMnSOD*, *G. hirsutum* MnSOD gene; *GhCAT1*, *G. hirsutum* CAT subunit 1 gene; *GhPOD1*, *G. hirsutum* POD1 gene; *GhUBQ7*, *G. hirsutum* ubiquitin 7 gene; MDA, malondialdehyde; NAC, *N*-acetyl L-cysteine.

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productivity, plants have evolved antioxidant defense mechanisms, of which a well-known one is the antioxidant enzyme system. Superoxide dismutase (SOD; EC 1.15.1.1) is the major scavenger of O_2^- in this system. It converts O_2^- to H_2O_2, which can be scavenged by catalase (CAT; EC 1.11.1.6), ascorbate peroxidase (APX; EC 1.11.1.11) [8], and peroxidase (POD; EC 1.11.1.7) [9]. These antioxidant enzymes are responsible for maintaining ROS at an appropriate level in the cell.

High accumulation of ROS has been considered for many years to be an undesirable and harmful event in stress metabolism [10]. However, several lines of evidence have suggested that ROS also act as signaling molecules [11–17], especially hydrogen peroxide, which plays an important role in responses to abiotic stress conditions [18–21]. In plants, H_2O_2 is produced in mitochondria, chloroplasts, peroxisomes, and at the plasma membrane or cell wall [22]. By comparison, H_2O_2 is a relatively long-lived (1 ms) molecule that is transported either by aquaporins [23,24] or by direct diffusion across membranes [25]. This characteristic is compatible with its role as a signaling molecule in plant growth, development, stomatal closure, root gravitropism, and abiotic stress [12,26–30]. A corresponding study has identified 175 expressed sequence tags (EST) of which 113 ESTs were induced and 62 ESTs were repressed by H_2O_2 in *Arabidopsis thaliana* [31].

Cotton fiber is the most natural material used in the textile industry; however, its yield is severely reduced by soil salinity [32]. In previous studies, the relationship between salt tolerance and antioxidant enzymes in cotton has been extensively investigated. Under salt stress, the balance between production and quenching of ROS was broken, with salt-tolerant cultivars upregulating their anti-oxidative enzymes at the cellular [33,34] or plant levels [35,36] more vigorously than salt-sensitive cultivars, suggesting that the antioxidant system was involved in the salt tolerance. There is a strong correlation between salt tolerance and antioxidant enzymes (such as CAT, POD, APX, and SOD), but the manner in which these enzymes are regulated in cotton remains unclear.

Cotton cultivar Xinluzhong 31 (XLZ31), used as the experimental material in our study, is a hybrid developed from *Gossypium hirsutum* × *G. herbaceum*in Xinjiang, China. In preliminary studies, this cultivar showed higher activity of antioxidant enzymes and salt tolerance than did others [37]. Our hypothesis was that salt-induced H_2O_2 in XLZ31, as a signaling molecule, activates the antioxidant system to alleviate oxidative stress on the membrane and improve salt tolerance. In this study, we investigated the role of H_2O_2 in regulating the activity of antioxidant enzymes and transcripts of these corresponding genes. The results suggested that H_2O_2 might modulate APX and SOD activities by upregulating the transcription of the *GhcAPX1*, *GhchlCSD*, and *GhFeSOD* genes in cotton to improve salt tolerance.

2. Materials and methods

2.1. Plant materials and treatments

Seeds of cotton cultivar XLZ31 were sown in pots containing perlite:vermiculite (1:3) in a 16 h:8 h light/dark cycle under a temperature regime of 20–29 °C and 40–60% relative humidity. Seedlings after emergence for 1 week were transferred to a 250 mL bottle containing 100 mL Hoagland solution (pH 6.0). Nutrient solution was added every other day and replaced every week. After 20-day cultivation, young plants with four leaves and growth vigor were transferred into nutrient solution containing 200 mmol L^{-1} NaCl, 200 mmol L^{-1} NaCl with NAC (0, 1, and 5 mmol L^{-1}), or H_2O_2 (0, 0.05, 0.1, 0.5, 1, and 10 mmol L^{-1}) for 24 or 12 h. Leaves at the same positions on cotton seedlings were harvested in three replicates and stored in liquid nitrogen for later use.

2.2. H_2O_2 and lipid peroxidation determination

Measurement of H_2O_2 followed Hu et al. [38] and Xue et al. [39]. Fresh leaves (200 mg) were homogenized in 2 mL cold acetone and centrifuged at 12,000×*g* for 10 min at 4 °C. Then 1 mL of supernatant was transferred into a 1.5-mL microtube containing 0.1 mL titanium sulfate (5%, w/v) and 0.2 mL strong aqua ammonia. After thorough mixing and centrifugation at 9,000×*g* for 5 min, the precipitate was washed with acetone twice or more until no chlorophyll remained. The resulting titanium–peroxide complex was dissolved in 5 mL H_2SO_4 (2 mol L^{-1}). The content of H_2O_2 was measured by the absorbance at 415 nm. The amount was calculated from a standard curve of H_2O_2 and expressed as nmol g^{-1} fresh weight. Lipid peroxidation was estimated from the formation of malondialdehyde (MDA), which was assayed according to Parida et al. [40].

2.3. Determination of enzyme activities

To extract total protein, 200 mg fresh leaves were homogenized in liquid nitrogen, and 2 mL extraction buffer was added. The buffer contained 50 mmol L^{-1} sodium phosphate (pH 7.8), 0.5 mmol L^{-1} EDTA, 1% polyvinylpyrrolidone-40 (PVP, w/v), 1 mmol L^{-1} ascorbic acid (ASA), and 20% glycerol (v/v). The homogenate was centrifuged at 15,000×*g* for 15 min, and the resulting supernatant was immediately used for the enzyme assay [41].

APX activity was measured by oxidation of ASA at 290 nm, following Tseng et al. [41] and Batisha et al. [42]. Some changes were made as follows: 3 mL reaction mixture contained 50 mmol L^{-1}sodium phosphate buffer (pH 7.0, 1.8 mL), 15 mmol L^{-1} ASA (0.1 mL), 0.3 mmol L^{-1} H_2O_2 (1 mL), and 0.1 mL enzyme extract. CAT activity was determined spectrophotometrically by H_2O_2 destruction at 240 nm following Jung [43]. The reaction mixture (3 mL) contained 1.95 mL deionized water, 100 mmol L^{-1} H_2O_2 (1 mL), and 50 μL enzyme extract. POD activity was estimated following Kim et al. [44]. The reaction buffer contained 100 mmol L^{-1} H_2O_2 (1 mL), 0.2% guaiacol (1 mL, w/v), 50 mmol L^{-1} sodium phosphate buffer (pH 7.0, 0.95 mL), and 50 μL enzyme extract. The activity of SOD was determined by the inhibition of photochemical reduction of nitro blue tetrazolium following Desingh and Kanagaraj [36].

2.4. mRNA quantification by quantitative real-time PCR (qRT-PCR)

Total RNA was isolated from 100 mg fresh leaves of each sample using an RNA kit (Bioteke, Beijing, China) according to the instructions of the manufacturer. After digestion with DNaseI

(TaKaRa, Dalian, China), approximately 2 μg of RNA was reverse-transcribed to cDNA using a random primer (TaKaRa) and Reverse Transcriptase M-MLV (RNase H⁻, TaKaRa).

qRT-PCR was performed with SYBR GREEN I using an SYBR Premix EX *Taq* II perfect Real time kit (TaKaRa) on a Gene Amp 5700 Sequence Detector (PE Applied Biosystems, CA, USA). Samples were analyzed in triplicate using independent RNA samples. Each reaction was performed in a total volume of 25 μL, containing 12.5 μL SYBR Premix Mix, 0.4 μmol L^{-1} of each primer (Table 1), and 2 μL cDNA, corresponding to 100 ng RNA. The following amplification program and calculation followed Sung et al. [45]. Dissociation curves were plotted after the PCR reaction, and the CT value of each replicated sample was the mean value from four assays. The $2^{-\Delta\Delta CT}$ method was used to calculate the relative change in mRNA level, which was normalized to a reference gene (*GhUBQ7*), and the fold increase was calculated relative to RNA sample without NaCl or H_2O_2 treatment. The full-length cDNAs of *GhMnSOD* (DQ088820), *GhFeSOD* (DQ088821), *GhchlCSD* (DQ088819), *GhcAPX1* (EF432582), *GhPOD1* (AJ606075), *GhCAT1* (X52135) were used for primer design. To standardize the results, *GhUBQ7* (AY189972) was used as the internal standard and the relative abundance was determined [46].

2.5. Statistic analysis

Statistics were analyzed with GraphPad Prism 4.0 (GraphPad Software, CA, USA). The data were means of at least three replicates and analyzed by one-way analysis of variance. The differences were tested by *t*-test, with *P*-values less than the 0.05 considered significant.

3. Results

3.1. Effects of NaCl treatment on H_2O_2 level in cotton plants

To investigate the role of H_2O_2 under salt stress, the accumulation of H_2O_2 in leaves was determined spectrophotometrically. Upon 200 mmol L^{-1} NaCl treatment, the concentration of H_2O_2 was stimulated within 24 h and reached a maximum at 12 h (Fig. 1A). Although H_2O_2 concentration showed a slight decrease during the second 12 h of treatment, the level was still significantly higher than the control ($P < 0.05$). As shown in Fig. 1B, no significant change in MDA concentration was observed in this experiment, indicating that the accumulation of H_2O_2 was not high enough to cause oxidative damage in this situation.

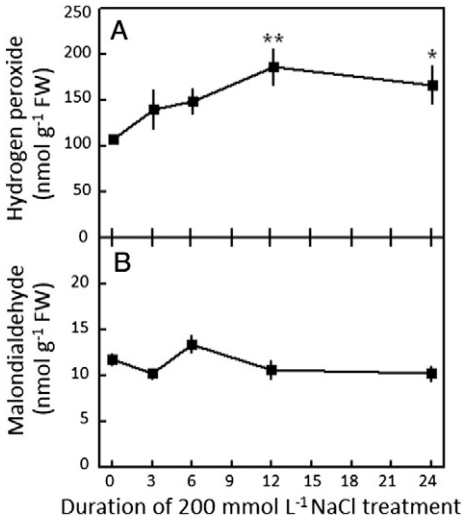

Fig. 1 – Effect of 200 mmol L^{-1} NaCl on H_2O_2 content (A) and MDA (B) content in cotton over 24 h. Values are means ± SD, *n* = 3, and asterisk indicates significant difference by *t*-test compared to control. *Significant at $P < 0.05$. **Significant at $P < 0.01$.

3.2. Effects of NaCl treatment on activities of antioxidant enzymes and transcription levels of corresponding genes

Salt stress stimulated the activities of CAT, APX, POD, and SOD, but time-course patterns varied among enzymes. The activities of CAT, POD, and SOD rapidly increased and reached maximum values within 3 h after exposure to 200 mmol L^{-1} NaCl (Fig. 2A, C, and D), suggesting that CAT, POD, and SOD are fast responders and may act as first-line defenses against salt stress. The activities of CAT, POD, and SOD remained stable for 24 h except for CAT and SOD at 6 h of treatment. APX activity was also increased and reached a maximum at 12 h after salt stress (Fig. 2B), suggesting that APX is a slow responder that clears ROS at a later time. These results suggest that different time-course patterns of antioxidant enzymes may facilitate the clearance of ROS.

As shown in Fig. 3, transcription levels of antioxidant enzymes genes were increased by 200 mmol L^{-1} NaCl over a 24-h period at different time points. The transcript levels of *GhCAT1* and *GhcAPX1* increased rapidly in the beginning 3 h but decreased during the next 3 h of treatment, followed by a steady increase (Fig. 3A, B). The expression level of *GhPOD1* was also elevated and reached a plateau after 6 h of salt stress

Table 1 – Quantitative real-time PCR primers for genes of antioxidant enzymes.

Gene	Forward primer (5′–3′)	Reverse primer (5′–3′)
GhchlCSDGhM	GCTCCCTCGTCAATCCCTCTGAATGCTGAGG	GTGACAACGCCTTCAACTTCCGCCAAGCAAAG
nSOD	GTGCTGGTT	GAACTAAATGTG
GhFeSOD	GCTTTCTCACTCGGGTCTGC	CACTTCCAATGCCCTCTTACTCA
GhcAPX1	ATGCTGCTAACAACGGCCTA	AGTAATCTCAACGGCAACGACA
GhCAT1	AGCCCAACCCTAAGTCCCATA	CACCCAAATCATCAAAGAGGAAAGT
GhPOD1	ATGTGAATGTCCGAATACCTCTTG	CCACTATTTGCCTCGTCATCCT
GhUBQ7	CTCGCAGTGCTCCAGTTCTAC	CACTTACCACAATAGTGCCTATCAA

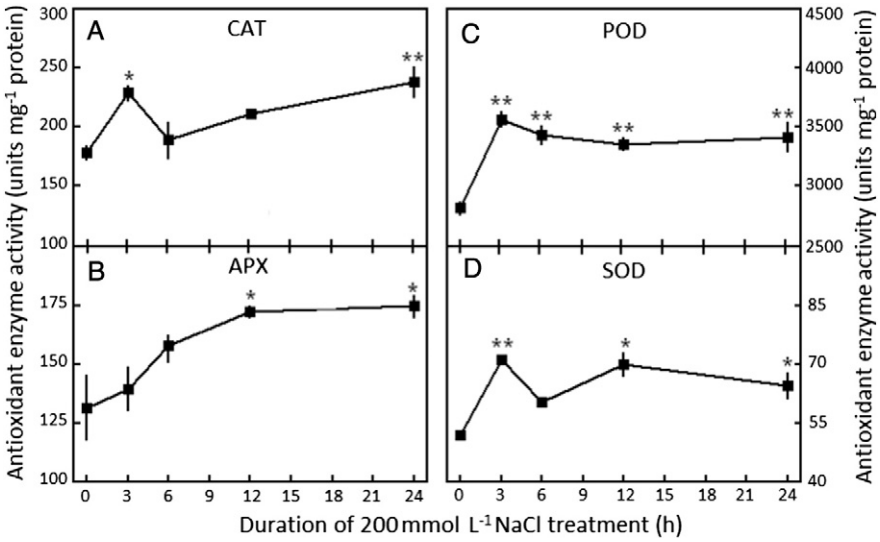

Fig. 2 – Time-course changes in activities of CAT (A), APX (B), POD (C), and SOD (D) in cotton in response to 200 mmol L^{-1} NaCl for 24 h. Values are means ± SD, *n* = 3, and asterisk indicates significant difference by *t*-test compared to control.

(Fig. 3C). Although the transcription levels of three genes encoding SOD did not rise as much as *GhCAT1*, the mRNA level of *GhMnSOD* still increased by two fold over the control at 3 h, and the maximum *GhchlCSD* and *GhFeSOD* expression levels were also significantly higher than the control ($P < 0.05$) (Fig. 3D–F). The patterns of change in transcription levels were generally consistent with the dynamic changes of corresponding enzyme activities.

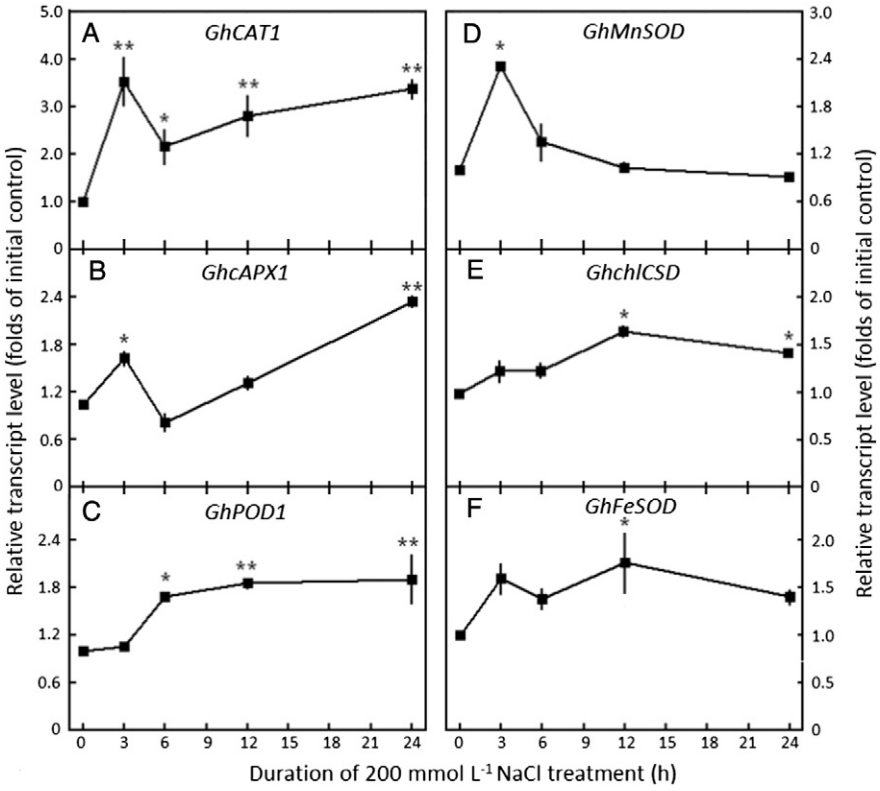

Fig. 3 – Time-course changes in the relative transcripts level of *GhCAT1* (A), *GhcAPX1* (B), *GhPOD1* (C), *GhMnSOD* (D), *GhchlCSD* (E), and *GhFeSOD* (F) of cotton in response to 200 mmol L^{-1} NaCl for 24 h. Values are means ± SD, *n* = 3, and asterisk indicates significant difference by *t*-test compared to control.

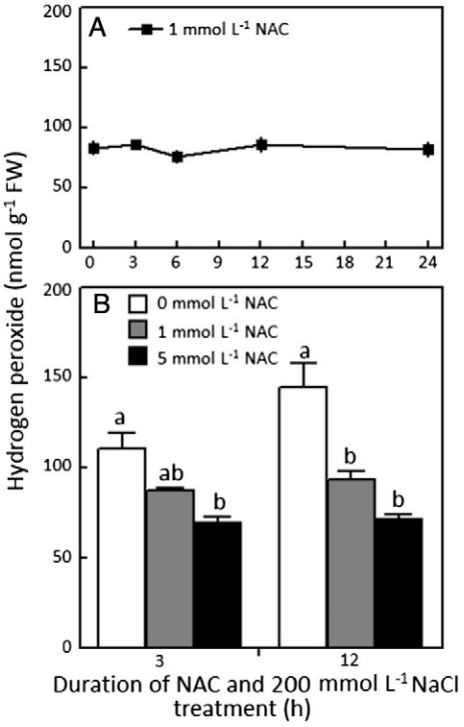

Fig. 4 – Changes in H_2O_2 content in response to 1 mmol L^{-1} additional NAC as time-course advanced (A) or to NAC (0, 1, and 5 mmol L^{-1}) at hours 3 and 12 (B) under 200 mmol L^{-1} NaCl treatment. Values are means ± SD, n = 3, and different symbols indicate significant difference at $P < 0.05$.

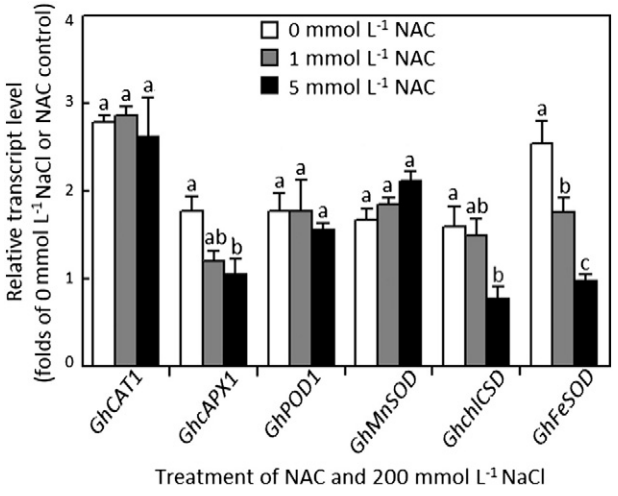

Fig. 5 – Effect of NAC and 200 mmol L^{-1} NaCl on relative transcript levels of genes encoding antioxidant enzymes in cotton. The effect of NAC on genes was assayed at hour 12 except *GhcAPX1* and *GhMnSOD*, whose transcripts reached maxima more rapidly than the others within the first 12 h, so that they were assayed at hour 3. Values are means ± SD, n = 3, and different symbols indicate significant difference at $P < 0.05$.

To elucidate the function of H_2O_2 in the regulation of antioxidant enzymes and the corresponding genes' transcripts, an H_2O_2 scavenger (NAC) was applied under 200 mmol L^{-1} NaCl treatment. In the presence of 1 mmol L^{-1} NAC, the increase of H_2O_2 was inhibited (Fig. 4A). Furthermore, the effect of inhibition was dose-dependently enhanced at both hours 3 and 12 of treatment (Fig. 4B). This result suggested that H_2O_2 induced by 200 mmol L^{-1} NaCl was effectively scavenged by NAC. The activities of APX and SOD were significantly repressed by NAC treatment, while the activity of CAT or POD was not changed (Table 2). Under 200 mmol L^{-1} NaCl treatment, NAC dose-dependently reduced the transcription levels of *GhcAPX1*, *GhchlCSD*, and *GhFeSOD* to 38%, 48%, and 59%, but not those of *GhMnSOD*, *GhCAT1*, and *GhPOD1* (Fig. 5). These results suggested that H_2O_2 directly regulated the expression of *GhcAPX1*, *GhchlCSD*, and *GhFeSOD*.

Table 2 – Effect of NAC and 200 mmol L^{-1} NaCl on the activities of antioxidant enzymes.

NAC	Enzymes activity (U mg^{-1} protein)			
	SOD	APX	CAT	POD
0 mmol L^{-1}	62.57 a	166.8 a	207.6 a	3356 a
1 mmol L^{-1}	38.90 b	150.2 a	173.4 b	3164 a
5 mmol L^{-1}	35.48 b	113.5 b	185.6 ab	3171 a

The activities of enzymes were determined after 12 h. Values are means of three replicates. In each column, values represented by the same letter are not significantly different at $P < 0.05$.

3.3. *Effects of exogenous H_2O_2 on antioxidant enzyme activities and transcription levels*

To evaluate the response of antioxidant enzymes to H_2O_2 stimulation, cotton plants of the same age as in the above experiment were treated with several concentrations of H_2O_2 (0, 0.05, 0.1, 0.5, 1, and 10 mmol L^{-1}), and leaves were collected at 12 h after treatment. MDA content was significantly increased under higher levels of H_2O_2 (0.5, 1.0, and 10.0 mmol L^{-1}) (Fig. 6), suggesting that high concentrations of H_2O_2 caused the peroxidation of lipids.

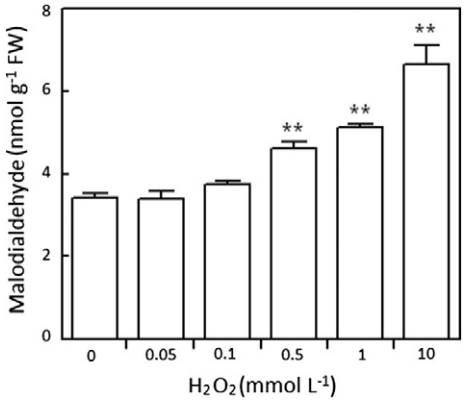

Fig. 6 – Effect of exogenous H_2O_2 on MDA content. Values are means ± SD, n = 3, and asterisk indicates significant difference by t-test compared to control at $P < 0.05$.

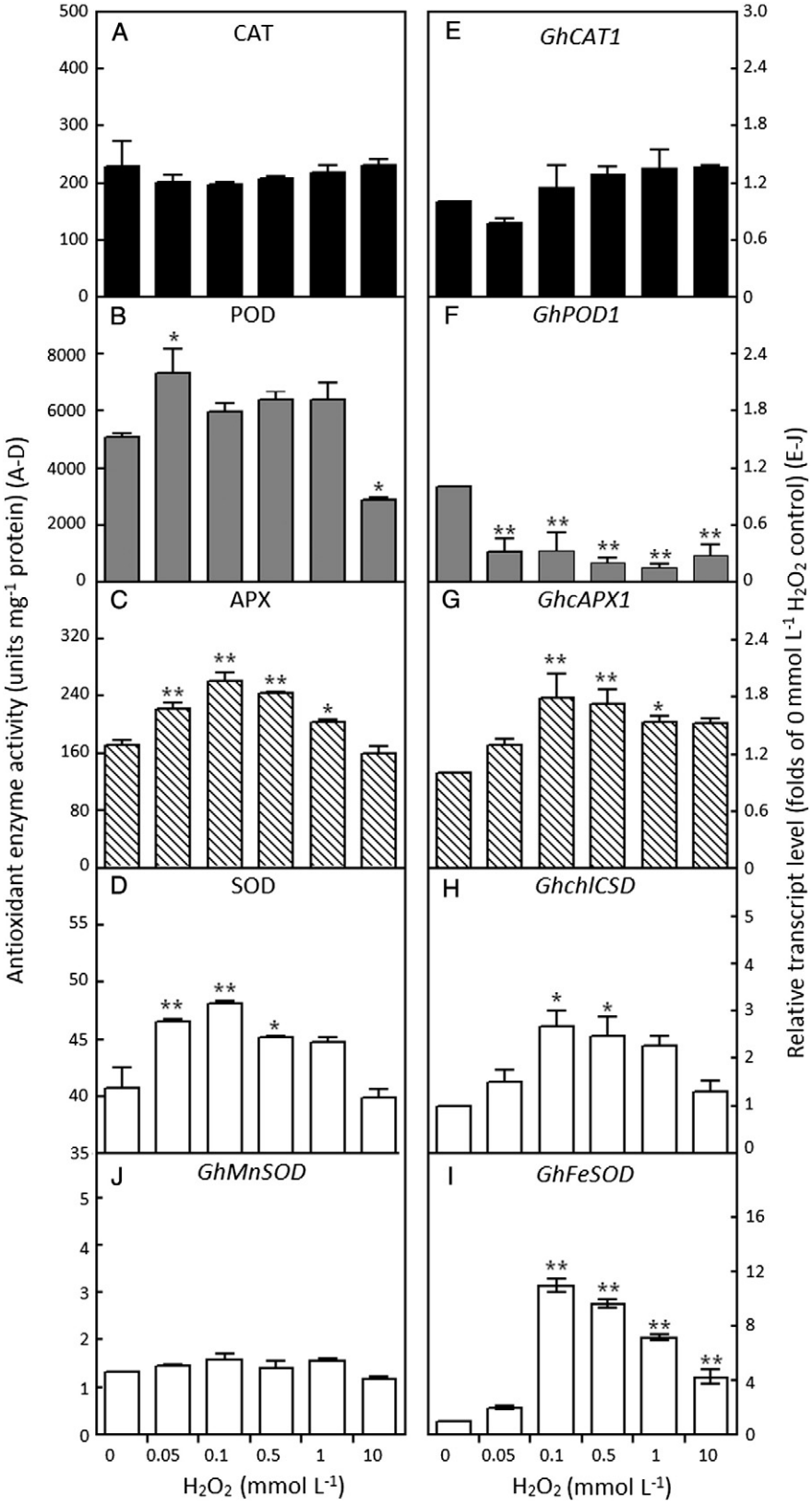

Fig. 7 – Effect of exogenous H_2O_2 on the activities of CAT (A), POD (B), APX (C), and SOD(D) and relative transcript levels of *GhCAT1* (E), *GhPOD1* (F), *GhcAPX1* (G), *GhchlCSD* (H), *GhFeSOD* (I), and *GhMnSOD* (J) after 12-h treatment. Values are means ± SD, *n* = 3, and asterisk indicates significant difference by *t*-test compared to control.

The activities of antioxidant enzymes and the levels of their gene expression were detected at different concentrations of H_2O_2 treatment. The activity and transcriptional level of CAT were not affected by H_2O_2 treatment, which was different from that of the NaCl treatment (Fig. 7A, E). The activities of POD, APX, and SOD were dose-dependently increased by H_2O_2 treatment (Fig. 7B–D). The transcription levels of *GhcAPX1*, *GhchlCSD*, and *GhFeSOD* were also dose-dependently upregulated by H_2O_2 treatment, responses consistent with the activities of the corresponding enzymes (Fig. 7G–I). Surprisingly, the expression level of *GhPOD1* was inhibited under H_2O_2 treatment (Fig. 7F).

Given that both APX and SOD activities and transcriptional levels of *GhcAPX1*, *GhchlCSD*, and *GhFeSOD* were dose-dependently increased by H_2O_2 treatment, we investigated whether the increased activities were correlated with the upregulated transcriptional levels of their genes. We found that APX activity was significantly correlated with the transcription level of *GhcAPX1* ($r = 0.593$, $P < 0.01$), and that SOD activity was significantly correlated with the transcription levels of *GhchlCSD* ($r = 0.584$, $P < 0.01$) and *GhFeSOD* ($r = 0.517$, $P < 0.05$). These results suggest that the upregulated transcription levels of *GhcAPX1*, *GhchlCSD*, and *GhFeSOD* may promote enzyme activities, improving salt and oxidative tolerance.

4. Discussion

In response to stress stimuli, the accumulation of H_2O_2 in plants is often observed [2,29,39,47]. In this study, we also found that H_2O_2 was accumulated in cotton cultivar XLZ31 under NaCl treatment (Fig. 1). It has been suggested that H_2O_2 is a signaling molecule in defense and adaptive responses [18,20,48–51]. The enhancement of H_2O_2 generation was followed by increases in the activities of antioxidant enzymes in plants [52–57]. Consistent with these findings, we observed that both NaCl-induced and exogenous H_2O_2 could increase antioxidant enzyme activities and transcriptional levels of the corresponding genes (Figs. 2, 3, and 7). It is well known that CAT, APX, POD, and SOD are key enzymes for ROS scavenging in plants. Significant increases in activities of these enzymes were often observed in salt-tolerant cotton cultivars exposed to NaCl for months [33]. In the present study, the activities of CAT, APX, POD, and SOD were significantly increased by 200 mmol L^{-1} NaCl treatment within 24 h (Fig. 2). However, MDA content was unchanged under salt stress (Fig. 1B), suggesting that oxidative attack might be effectively alleviated by increased activities of antioxidant enzymes. Generally, increased activities of antioxidant enzymes are correlated with the upregulated transcriptional levels of the corresponding genes (Figs. 2, 3). Time-course changes in transcripts of *GhCAT1*, *GhcAPX1*, *GhPOD1*, *GhMnSOD*, *GhchlCSD*, and *GhFeSOD* revealed that these genes were induced to provide transcripts of enzymes. However, there was some discrepancy between enzyme activity and transcription level, such as shown by APX activity and *GhcAPX1* transcription (Fig. 2B, 3-B). This phenomenon is probably due to the encoding of antioxidant enzymes by a multigene family, or to the influence on expression levels of other factors such as posttranscriptional regulation [58–60]. The immediate increase of POD activity after 3 h treatment without mRNA increase (Figs. 2C and 3C) suggested that the early increase might be under translational regulation for rapid modulation of activity [45].

We further found that the activities of antioxidant enzymes and corresponding transcriptional levels of antioxidant genes were inhibited by the H_2O_2 scavenger NAC under salt stress (Figs. 4, 5). Although O_2^- production could also be scavenged by NAC [13], the O_2^- level was not reduced in our experiment (data not shown), suggesting that the counteracting effect of NAC was H_2O_2 specific in this experiment. These results indicated that the changes of antioxidant-related gene transcript levels and enzyme activities in cotton were tightly regulated by the accumulation of H_2O_2 under salt stress. However, the expression levels and the activities of CAT and POD were not affected by NAC or exogenous H_2O_2 (Fig. 5, Table 2, Fig. 7A, B, E, F). This discrepancy indicated that the expression levels and activities of CAT and POD were regulated by other factors induced by NaCl treatment, a finding inviting further investigation.

We found that the MDA content and its correlation with exogenous H_2O_2 level ($r = 0.88$, $P < 0.01$) (Fig. 6) reflected the oxidative damage in cotton when exposed to higher concentrations of H_2O_2 (0.5, 1.0, and 10.0 mmol L^{-1}, but that stress induced by lower concentrations of H_2O_2 (0.05 and 0.10 mmol L^{-1}) could be effectively prevented. This finding is consistent with the dual role of H_2O_2 in plants: at higher levels, it leads to oxidative stress and even triggers cell death [11,18,61], while at lower levels, it acts as a signal molecule mediating responses to various stresses [15,27,62,63]. Consistently, the activity of APX or SOD was induced by lower concentrations of H_2O_2 but suppressed by higher levels of H_2O_2 (Fig. 7C, D). The same result was observed for the transcriptional levels of *GhcAPX1*, *GhchlCSD*, and *GhFeSOD* (Fig. 7G–I). The expression of *FeSOD* seemed to be modulated more tightly by H_2O_2 than did that of other isoenzymes (Fig. 7I). The same phenomenon was also observed in *Marchantia polymorpha* L. [44], possibly owing to the affinity between H_2O_2 and iron [64,65]. Taken together, these results suggest that H_2O_2 plays an important role in the regulation of the expressions and activities of antioxidant enzymes that lead to increased salt tolerance.

5. Conclusion

In conclusion, NaCl-induced and exogenous H_2O_2 regulate the activities of antioxidant enzymes as well as the transcription levels of corresponding genes in cotton. APX and SOD activities were significantly correlated with the transcriptional levels of *GhcAPX1* and *GhchlCSD/GhFeSOD*, respectively, under salt stress. These results suggest that H_2O_2 may act as a second messenger modulating antioxidant gene expression and enzyme activity and thereby increasing salt tolerance.

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